Gamma Radiation Inactivation of Enterococci

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ABSTRACT

Radiation survival curves were determined for 7 strains of *Enterococcus faecium*, 10 strains of *E. faecalis*, and 8 strains of the proteolytic variety of *E. faecalis*. The D values (i.e. the doses giving 90% reduction of viable counts) ranged from 0.5-4.7 kGy for the *E. faecium* strains; 0.35-2.1 kGy for the *E. faecalis* strains; and 0.3-0.45 kGy for the proteolytic variants of *E. faecalis*. The survival curves were linear for most strains but some exhibited significant non-linear trends.

Enterococci are streptococci that inhabit the intestinal tracts of mammals and possess the group D antigen. Some members of the group *(Streptococcus bovis* and *S. equinus)* do not survive apart from their respective hosts and are not associated with most common foods. The most important members of the group D streptococci that are routinely found in foods are *Enterococcus faecalis* and *E. faecium.* These two species occur commonly in nature and have been isolated from wild plants *(6)*, domestic plants, and insects *(6,10).* They can also be routinely isolated from the intestinal tracts of many animals *(8,11,13)* as well as from finished product (e.g. bacon) as shown by Cavett *(3).*

Concern has been expressed that low dose irradiation (i.e. less than 1 Mrad or 10kGy) could selectively destroy radiation sensitive bacteria in foods that may normally inhibit pathogenic bacteria including *C. botulinum (2,16).* Inhibition of *C. botulinum* by indigenous bacteria in pork was demonstrated in one study *(9)* which also showed that when the meat was irradiated at a dose of 7.5 kGy, toxin production was more rapid than in nonirradiated controls. This was the result of inhibition of acid-producing indigenous bacteria. The results of another study, moreover, showed that the addition of a radiation resistant *E. faecalis* culture isolated from irradiated pork prevented the formation of botulinal toxin in similarly irradiated samples *(7)*; enough acid was produced by surviving *E. faecalis* cells to inhibit toxin formation by *C. botulinum.*

The present study was initiated to determine the radiation sensitivities of other strains of enterococci with the objective of utilizing any radiation resistant strains for inhibiting pathogenic bacteria in foods that may be irradiated at low doses.

MATERIALS AND METHODS

Cultures

The enterococcus cultures were obtained from several sources (Table 1). Some of them were identified as species of the genus *Streptococcus*; others were described only as group D streptococci. These strains would now probably be classified as *Enterococcus* strains [Facklam and Collins *(5)*; Schleifer and Kilpper-Bälz *(14)*]. The cultures were maintained on slants of plate count agar (Difco) and were kept at 4°C. For the irradiation studies, the organisms were transferred to a buffered organic medium (BNT) consisting of 0.4 g nutrient broth (Difco), 1.5 g trypticase soy broth with glucose (BBL), 0.026 M KH_2PO_4, and 0.028 M $Na_2HPO_4.7H_2O$. The buffer maintained the pH between 6.4-6.6 during growth. The incubation temperature was 35°C. Maximum growth temperatures were determined by incubating BNT tubes in a water bath (Precision model 260) with the temperature monitored with two thermistors (YSI model 42SC). Growth in the presence of 0.04% K tellurite was determined in BNT medium incubated 2 weeks at 35°C; acid production from sugars was determined in purple broth base (Difco) by using separately autoclaved sugar solutions at final concentrations of 0.5%

Irradiation

For irradiation, 1-ml portions were transferred to 3 ml-capacity vials and screw caps were securely fastened. These were placed in an ice water bath prior to irradiation and were kept at a temperature of 0-2°C during irradiation by the use of the gaseous phase of liquid nitrogen. A ^{137}Cs source providing a dose rate of 125 Gy/min was the radiation source.

Culture enumeration

After irradiation, the samples were diluted with sterile water at a temperature of 4-7°C. A spiral plater and colony counter (Spiral Systems, Inc.) were used to determine population densities, using plate count agar incubated 2 d at 35°C. The plate counts were converted to $\log_{10}$ and the best fitting regression lines with their correlation coefficients and slopes were calculated by using Sigma-Plot version 3.10 (Jandel Scientific). Standard deviations of the log transformed means were used as the error bars for the graphs. Radiation D values were calculated by dividing the applied dose in kGy by the difference in $\log_{10}$ counts of the non-irriadiated and irradiated samples (this gives the same result as the reciprocal of the slope of the linear regression line). Although some survival curves were not linear, D values were approximated from linear representations of the data.

TABLE 1. *Characteristics of enterococcus cultures.*

Source[a]	Number	Species[b]	Melezitose	Acid from Melibiose	Sucrose	Max temp[c]
ERRC	22-1	*E. faecium*	-	+	+	48.6
ERRC	22-2	*E. faecium*	-	+	+	48.6
ERRC	93-8	*E. faecium*	-	+	+	46.3
Kraft	B2906	*E. faecium*	-	+	+	47.8
Kraft	B2907	*E. faecium*	-	+	-	44.8
ATCC	25307	*E. faecium*	-	+	-	47.8
ATCC	19581	*E. faecium*	-	+	-	47.8
Kraft	B2904	*E. faecalis*	-	+	+	46.3
Cornell	76-1	*E. faecalis*	-	-	-	46.3
Cornell	19-1	*E. faecalis*	-	+	-	47.3
ATCC	19433	*E. faecalis*	+	-	+	46.3
ATCC	29212	*E. faecalis*	+	-	+	46.3
NRRC	B1295	*E. faecalis*	-	+	+	46.5
NRRC	B446	*E. faecalis*	-	+	+	47.8
ERRC	94-1	*E. faecalis*	-	+	+	47.3
ERRC	125-2	*E. faecalis*	-	+	+	46.3
NRRC	B537	*E. faecalis*	+	-	+	46.3
Kraft	B2905	*E. faecalis*[d]	+	-	+	44.8
Kraft	B2991	*E. faecalis*[d]	+	-	+	47.3
Kraft	B2908	*E. faecalis*[d]	+	-	+	46.3
Kraft	B2909	*E. faecalis*[d]	+	-	+	46.3
Cornell	T91	*E. faecalis*[d]	+	+	+	46.3
Cornell	D318	*E. faecalis*[d]	+	-	+	46.3
Cornell	76-2	*E. faecalis*[d]	+	-	+	46.3
Cornell	81	*E. faecalis*[d]	-	-	+	46.3

[a]Sources were: Kraft Co.; Eastern and Northern Regional Research Centers, USDA; American Type Culture Collection; and Cornell University; none formed pigment and all were non-motile.
[b]Classified from growth in 0.04% potassium tellurite (*E. faecium* negative).
[c]Cultures incubated 7 d at 44.8, 46.3, 47.3, 47.8, 48.6, and 49.0°C.
[d]These liquefied gelatin.

RESULTS AND DISCUSSION

Mundt *(12)* differentiated *E. faecium* from *E. faecalis* by their reaction to tellurite in organic media as well as on their ability to ferment melezitose and melibose; *E. faecalis* strains reportedly produce acid from melezitose but not from melibiose, whereas *E. faecium* produces opposite reactions. Table 1 indicates that the cultures classified as *E. faecium*, based on their inability to grow in the presence of 0.04% potassium tellurite, showed the expected reactions to these sugars, but cultures that grew in the presence of 0.04% potassium tellurite (thus classifying them as *E. faecalis*) showed variable responses. Acid production from these sugars by the proteolytic variety of *E. faecalis* was the most consistent in showing the expected reactions, only 1 of 8 strains showing an aberrant response to either melezitose or melibiose. None of the cultures grew at 49°C although the 8th edition of Bergeys Manual *(4)* indicated that *E. faecium* could grow at 50°C but *E. fecalis* could not. Mundt, in the 1986 Manual of Systematic Bacteriology *(12)*, did not include temperature growth maxima as differential characteristics but sugar fermentations were suggested as valuable criteria. However the results in Table 1 indicate that the fermentation of melezitose or melibiose do not provide clear differentiation of the two species. Fecklam and Collins *(5)* however, in a detailed study of clinical isolates of *E. faecalis* and *E. faecium* indicated that arabinose fermentation was a valuable differential characteristic; strains of *E. faecalis* were unable to ferment this sugar while *E. faecium* strains fermented it.

The D values in Table 2 indicate that there was considerable variation in radiation sensitivity of different strains within the two species and within the proteolytic strains of *E. faecalis*. The D value of 4.51 kGy for *E. faecium* ATCC 19581 indicates that this organism is more resistant than spores of the most resistant strain of *C. botulinum* to gamma radiation *(1)*. *E. faecalis* 94-1 isolated from irradiated pork is also quite resistant with a D value of 2.09 kGy; this is equivalent to the resistance of most *C. botulinum* spores *(1)*. Strain 94-1 was used in a study of the efficacy of adding a radiation-resistant acid-producing microorganism for preventing toxin formation by *C. botulinum* in fresh pork or bacon *(7)*. When fermentable sugar was present, no toxin developed from bacon inoculated with this organism even when radiation levels were as high as 7.5 kGy. In fresh pork, indigenous bacteria produced enough acid to inhibit *C. botulinum*, but these did not survive low dose irradiation. However, toxin production was prevented when strain 94-1 was incorporated into non-irradiated or irradiated meat. *E. faecium* ATCC 19581 was not used in that study, but it should also protect against toxin formation by *C. botulinum*.

The shape of the radiation survivor plots for the *E.*

TABLE 2. *Regression parameters for irradiation survivor curves of enterococci.*

Culture	a	b_1	b_2	R	D
E. faecium 22-1	8.89	-0.0047	-7.518E-6	0.9990	1.06
E. faecium 22-2	8.85	-0.0094	-1.625E-5	0.9982	0.70
E. faecium 93-8	8.72	-0.0025	-3.600E-5	0.9990	0.75
E. faecium B2906	9.13	-0.0049	-9.418E-6	0.9959	0.93
E. faecium B2907	8.76	-0.0097	-1.975E-5	0.9921	0.64
E. faecium ATCC 25307	8.85	-0.0096	-2.839E-6	0.9952	1.04
E. faecium ATCC 19581	8.28	-0.0022		0.9962	4.51
E. faecalis B2904	9.18	-0.0100	-8.913E-6	0.9859	1.00
E. faecalis 76-1	8.42	-0.0167		0.9638	0.60
E. faecalis 19-1	8.39	-0.0180		0.9822	0.55
E. faecalis ATCC 19433	9.26	-0.0194		0.9906	0.52
E. faecalis ATCC 29212	8.89	-0.0260		0.9986	0.38
E. faecalis B1295	8.85	-0.0087		0.9988	1.15
E. faecalis B446	9.02	-0.0135		0.9979	0.74
E. faecalis 94-1	8.83	-0.0048		0.9937	2.09
E. faecalis 125-2	9.08	-0.0092		0.9909	1.08
E. faecalis B537	8.95	-0.0203		0.9990	0.49
E. faecalis B2905 (proteolytic)	9.00	-0.0108		0.9994	0.92
E. faecalis B2991 (proteolytic)	9.16	-0.0171		0.9957	0.58
E. faecalis B2908 (proteolytic)	8.39	-0.0196	-4.375E-5	0.9805	0.51
E. faecalis B2909 (proteolytic)	9.34	-0.0226		0.9920	0.44
E. faecalis T91 (proteolytic)	8.86	-0.0250		0.9882	0.40
E. faecalis D318 (proteolytic)	9.08	-0.0332		0.9892	0.30
E. faecalis 76-2 (proteolytic)	8.93	-0.0284		0.9864	0.35
E. faecalis 81 (proteolytic)	8.97	-0.0293		0.9977	0.34

Notes: The genreal term for the linear equation was $y = a + b_1x$ and for the quadratic equation it was $y = a + b_1x + b_2x^2$. The irradiation D values were calculated from linear regression curves as the negative reciprocal of the slope b_1.

faecium strains is shown in Fig. 1, and the parameters for the regression equations are shown in Table 2. Quadratic regression lines gave the best fit for these strains with the exception of ATCC 19581 whose survivor plot was a straight line. The regression plots for most of the *E. faecalis* strains (Figs. 2 and 3) were also straight lines. Quadratic regression curves or curves with "shoulders" have been described for irradiated cultures by Silverman and Sinskey *(15)*. One explanation for this phenomenon is that these organisms have a mechanism for reparing radiation damage; thus at lower doses repair may occur almost as fast as inactivation. At higher doses, repair cannot keep pace with inactivation and the survival curve steepens. Another explanation for a non-linear inactivation curve is that some microorganisms are attacked at multiple sites by the high energy photons or by the free radicals produced from their interaction with water molecules *(15)*. It is also possible that a tendency for chain formation may account for the shoulders although microscopic examination of these strains did not show evidence of this; all cultures were predominatly paired cocci. The opposite type of irradiation survivor curve (type 3) where there is rapid initial destruction with subsequent tailing did not occur with the enterococci of this study.

The studies reported here indicate that there are other useful radiation resistant strains of enterococci beside *E. faecalis* 94-1 which may find application in low-dose irradiated foods for preventing toxin formation by pathogenic microorganisms such as *C. botulinum*. These studies indi-

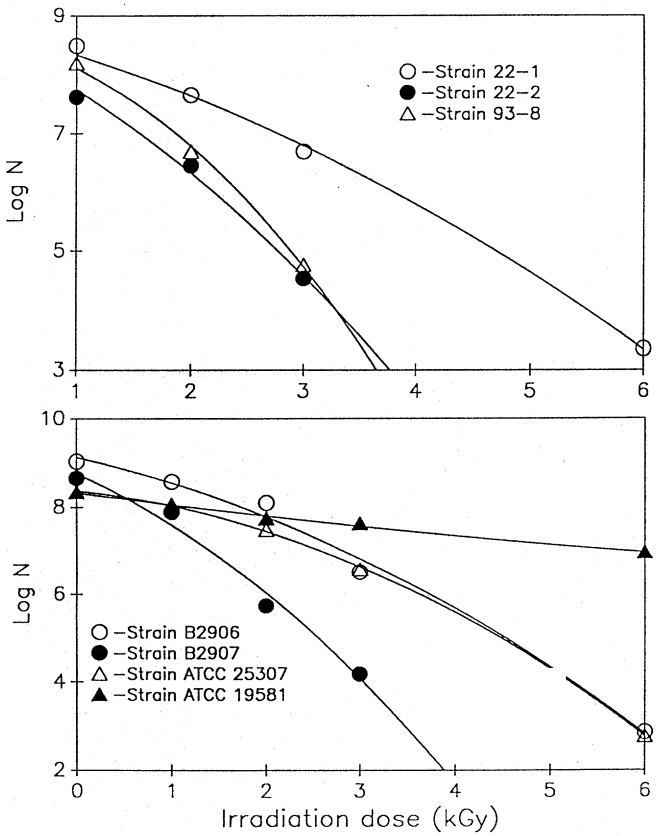

Figure 1. *Radiation sensitivities of* E. faecium *variants.*

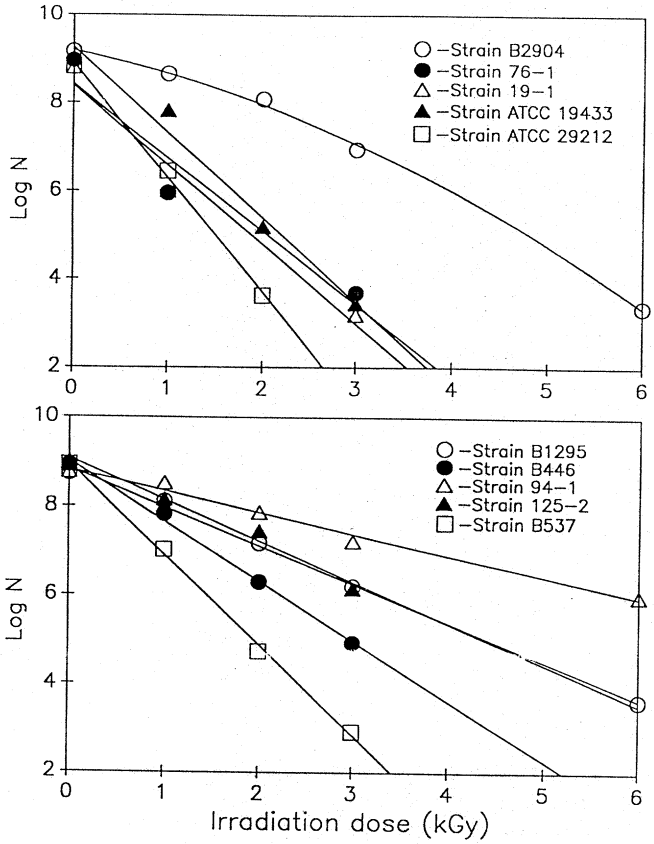

Figure 2. *Radiation sensitivities of* E. faecalis *variants.*

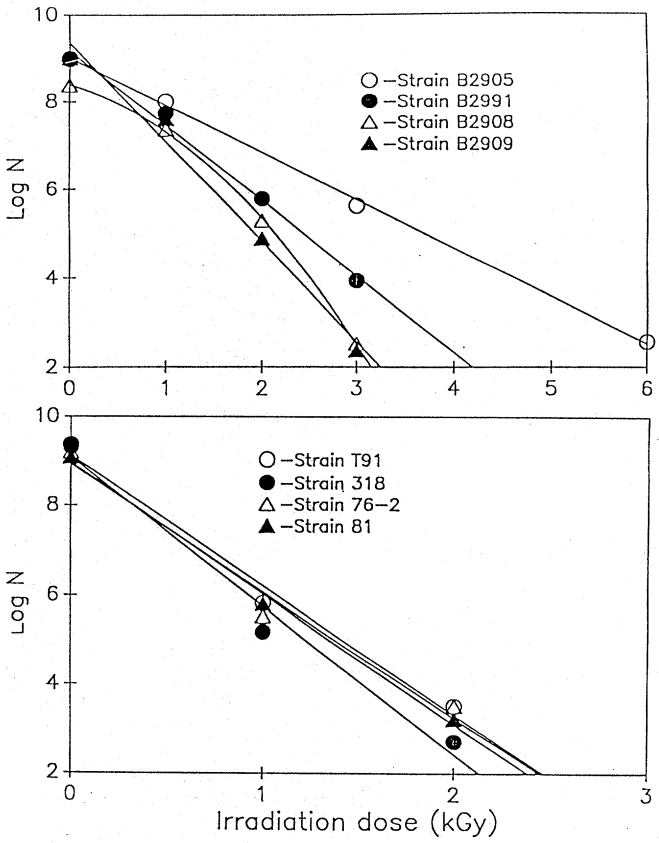

Figure 3. *Radiation sensitivities of the proteolytic variants of* E. faecalis.

cate that radiation sensitivity or resistance is not associated with the characteristics normally used for differentiating species of enterococci.

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